Immunofluorescence Detection of CD3 in Frozen Mouse Tissue

Reagent and Antibody Information

1X Wash Buffer
0.3% Hydrogen Peroxide
1% BSA Diluent
Normal Rabbit IgG – Affinity Purified
ProLong Gold Mounting Media

Blocking Serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 005-000-121

Avidin / Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # SP-2001

Primary Antibody: Rabbit Anti-CD3 Polyclonal Antibody
Abcam, Inc
Cambridge, MA 02139
www.abcam.com
1-888-772-2226
Catalog # ab5690

Secondary Antibody: Alexa Fluor® 488 Goat Anti-Mouse IgG (H+L) *****Highly Cross-Adsorbed*****
Life Technologies / Invitrogen
Grand Island, NY 14072
www.invitrogen.com
1-888-584-8929
Catalog # A-11209

Staining Procedure

Positive Control Tissue: Spleen – Cytotoxic T-cell lymphocytes
Stain Localization: Cell membrane and cytoplasmic

1. Cut each frozen section at 6µm and mount on a positively charged slide.
   Immediately fix the section in Rapid Fix Solution for 7 seconds.
   Rinse the slide thoroughly in tap water to remove excess fixative and then place in 1X Wash Buffer.
   Once all the slides have undergone this process, proceed to step 2.

2. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

3. Quench endogenous peroxidase by placing the slides in 0.3% hydrogen peroxide for 30 minutes.

4. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

5. Block with 10% normal goat serum for 20 minutes at room temperature.
   Lot #_____ Date Reconstituted_____

   DO NOT RINSE SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
   ONLY WIPE EXCESS BLOCK.

6. Apply the primary antibody at a 1:100 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date _____

   For negative control slides, dilute normal rabbit IgG so that it's IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:100 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date _____

7. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

From this point on, please perform the stain in the dark.

8. Apply the Alexa 488 goat anti-mouse secondary antibody at a 1:300 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date _____

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

10. Rinse the slides in tap water.

11. Coverslip with ProLong Gold Mounting Media (with or without DAPI).

12. Store slides at 4°C

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